

WHAT IS CLAIMED IS:

- 1 1. An oligonucleotide primer comprising in the following order from 5'
2 to 3':
3 a phage-encoded RNA polymerase recognition sequence,
4 a spacer sequence comprising a sequence of from 12 to 20 nucleotides
5 that consists of one nucleotide type or two different nucleotide
6 types, and
7 a target complementary sequence which can bind a segment of a target
8 nucleic acid.
- 1 2. The primer of claim 1, wherein said spacer sequence comprises a
2 nucleotide sequence having the formula $(XY)_n$,
3 wherein n is from 6 to 10,
4 wherein X and Y are independently selected from the group consisting of an
5 adenine nucleotide, a guanine nucleotide, a cytosine nucleotide, and a
6 thymidine nucleotide,
7 wherein X and Y are not the same.
- 1 3. The primer of claim 2, wherein X is an adenine nucleotide and Y is a
2 guanine nucleotide.
- 1 4. The primer of claim 3, wherein n is 9.
- 1 5. The primer of claim 1, wherein said spacer sequence comprises a
2 nucleotide sequence having the formula $(X)_n$,
3 wherein n is from 12 to 20,
4 wherein X is selected from the group consisting of an adenine nucleotide, a
5 guanine nucleotide, a cytosine nucleotide, and a thymidine nucleotide.
- 1 6. The primer of claim 5, wherein n is 18.
- 1 7. A method of amplifying a target nucleic acid in an aqueous solution
2 with a first and a second primer, said method comprising:
 - 3 i.) transcribing an intermediate duplex with a phage-encoded RNA
4 polymerase to form a sense transcription product having a 5' end and a 3' end,

5 wherein said intermediate duplex comprises a double-stranded
6 molecule, wherein said double-stranded DNA molecule
7 comprises a first and a second strand,
8 wherein said first strand comprises in the following order from 5' to 3':
9 a phage-encoded RNA polymerase recognition sequence,
10 a first spacer sequence comprising a sequence of from 12 to 20
11 nucleotides that consists of one nucleotide type or two
12 different nucleotide types, and
13 a first target complementary sequence which can bind to a
14 segment of said target nucleic acid,
15 wherein said second strand comprises in the following order from 5' to
16 3':
17 a second target complementary sequence which can bind to a
18 segment of said target nucleic acid,
19 a second spacer sequence comprising a sequence of from 12 to
20 20 nucleotides that consists of one nucleotide type or
21 two different nucleotide types, and
22 a phage-encoded RNA polymerase recognition sequence,
23 wherein said transcribing takes place in the presence of Mn⁺⁺, with all
24 four dNTPs, and with those rNTPs represented in said first
25 spacer sequence;

26 ii.) hybridizing said second primer to said sense transcription product to
27 form a second primer-sense transcription product complex,
28 wherein said second primer comprises in the following order from 5'
29 to 3':
30 a phage-encoded RNA polymerase recognition sequence,
31 said second spacer sequence, and
32 said second target complementary sequence which can bind to
33 a 3' segment of said target nucleic acid;

34 iii.) extending said second primer-sense transcription product complex with
35 a Reverse Transcriptase that lacks RNaseH activity to form a first amplification duplex;

36 iv.) transcribing said first amplification duplex with a phage-encoded RNA
37 polymerase, in the presence of Mn⁺⁺, with all four dNTPs, and with those rNTPs represented
38 in said second spacer sequence, to form an antisense transcription product;

39 v.) hybridizing said first primer to said antisense transcription product to
40 form a first primer-antisense transcription product complex,
41 wherein said first primer comprises in the following order from 5' to
42 3':
43 a phage-encoded RNA polymerase recognition sequence,
44 said first spacer sequence, and
45 said first target complementary sequence which can bind to a
46 5' segment of said target nucleic acid;
47 vi.) extending said second primer-antisense transcription product complex
48 with a Reverse Transcriptase that lacks RNaseH activity to form a second amplification
49 duplex; and
50 vii.) transcribing said second amplification duplex with a phage-encoded
51 RNA polymerase, in the presence of Mn⁺⁺, with all four dNTPs, and with those rNTPs
52 represented in said first spacer sequence to form said sense transcription product.

1 8. The method of claim 7, wherein the method further comprises
2 repetitively carrying out steps i to vii.

1 9. The method of claim 7, wherein said first or said second spacer
2 sequence comprises a nucleotide sequence having the formula (XY)_n,
3 wherein n is from 6 to 10,
4 wherein X and Y are independently selected from the group consisting of an
5 adenine nucleotide, a guanine nucleotide, a cytosine nucleotide, and a
6 thymidine nucleotide,
7 wherein X and Y are not the same.

1 10. The method of claim 9, wherein X is an adenine nucleotide and Y is a
2 guanine nucleotide.

1 11. The method of claim 10, wherein n is 9.

1 12. The method of claim 10, wherein the rNTPs are rATP and rGTP.

1 13. The method of claim 7, wherein said first or said second spacer
2 sequence comprises a nucleotide sequence having the formula (X)_n,
3 wherein n is from 12 to 20,

4 wherein X is selected from the group consisting of an adenine nucleotide, a
5 guanine nucleotide, a cytosine nucleotide, and a thymidine nucleotide.

1 14. The method of claim 13, wherein n is 18.

1 15. The method of claim 7, wherein said sense and antisense transcription
2 products comprise a nucleic acid strand comprising both ribonucleotides and
3 deoxyribonucleotides.

1 16. The method of claim 7, wherein said first and said second
2 amplification duplexes consist of deoxyribonucleotides and ribonucleotides.

1 17. The method of claim 7, wherein said method is carried out at a single
2 temperature.

1 18. The method of claim 7, wherein said method is carried out at a single
2 temperature of between 25 °C and 55 °C.

1 19. The method of claim 1, wherein the method is carried out at a single
2 temperature of greater than 50 °C.

1 20. The method of claim 7, wherein said intermediate duplex comprises a
2 double-stranded DNA comprising one complete primer sequence followed by the entire
3 sequence that is to amplified.

1 21. The method of claim 7, wherein said intermediate duplex is formed
2 from double-stranded DNA, single-stranded DNA, or RNA.

1 22. The method of claim 7, wherein said intermediate duplex is formed by
2 the process comprising the following steps of:

3 denaturing a double-stranded DNA target to form an upper strand and a lower
4 strand;

5 hybridizing said first primer to said lower strand to form a first primer-lower
6 strand complex;

7 extending said first primer-lower strand complex with a Reverse Transcriptase
8 that lacks RNaseH activity or with a DNA Polymerase to form a first long sense strand
9 product-lower strand complex;

10 denaturing said first long sense strand product-lower strand complex into a
11 first long sense strand product and said lower strand;
12 hybridizing said second primer to said first long sense strand product to form a
13 second primer-first long sense strand product; and
14 extending said first primer-first long antisense strand product with a Reverse
15 Transcriptase that lacks RNaseH activity or with a DNA Polymerase to yield said
16 intermediate duplex.

1 23. The method of claim 7, wherein said intermediate duplex is formed by
2 the process comprising the following steps of:

3 denaturing a double-stranded DNA target to form an upper strand and a lower
4 strand;

5 hybridizing said first primer to said lower strand to form a first primer-lower
6 strand complex;

7 extending said first primer-lower strand complex with a Reverse Transcriptase
8 that lacks RNaseH activity or with a DNA Polymerase to form a first long sense strand
9 product-lower strand complex, wherein said first long sense strand product has a 5' and a 3'
10 end;

11 displacing said first sense strand product from said lower strand by:

12 hybridizing a bumper oligonucleotide to a subsequence on said lower
13 strand adjacent to said 5' end of said first sense strand product
14 on the first sense strand product-lower strand complex;

15 extending said bumper oligonucleotide with a Reverse Transcriptase
16 that lacks RNaseH activity or with a DNA Polymerase,
17 thereby displacing said first sense strand product;

18 hybridizing said second primer to said first long sense strand product to form a
19 second primer-first long sense strand product; and

20 extending said first primer-first long antisense strand product with a Reverse
21 Transcriptase that lacks RNaseH activity or with a DNA Polymerase to yield said
22 intermediate duplex.

1 24. The method of claim 7, wherein said intermediate duplex is formed by
2 the process comprising the following steps of:

1 25. The method of claim 7, wherein said intermediate duplex is formed by
2 the process comprising the following steps of:
3 hybridizing said second primer to a single-stranded target RNA molecule to
4 form a second primer-RNA template complex;
5 extending said second primer-RNA template complex with a Reverse
6 Transcriptase that lacks RNaseH activity or a DNA Polymerase to form a first long antisense
7 strand product-template complex;
8 denaturing said first long antisense strand product-RNA template complex into
9 a first long antisense strand product and said single-stranded RNA molecule;
10 hybridizing said first primer to said first long antisense strand product to form
11 a first primer-first long antisense strand product complex; and
12 extending said first primer-first long antisense strand product with a Reverse
13 Transcriptase that lacks RNaseH activity or with a DNA Polymerase to yield said
14 intermediate duplex.

1 26. The method of claim 7, wherein said phage-encoded RNA
2 polymerase is polymerase selected from the group consisting of : a T7 RNA polymerase,
3 a T4 RNA polymerase, a T3 RNA polymerase, a SP6 RNA polymerase and a K11 RNA
4 polymerase.

1 27. The method of claim 26, wherein said phage-encoded RNA
2 polymerase is a mutant phage-encoded RNA polymerase that is competent to incorporate
3 dNTPs into a template nucleic acid.

1 28. The method of claim 27, wherein said phage-encoded RNA
2 polymerase is a T7 RNA polymerase.

1 29. The method of claim 28, wherein said T7 RNA polymerase
2 contains a Y639F mutation.

1 30. The method of claim 28, wherein said T7 RNA polymerase
2 contains a S641A mutation.

1 31. The method of claim 28, wherein said T7 RNA polymerase
2 contains at least two mutations.

1 32. The method of claim 7, wherein said Mn⁺⁺ is present in a
2 concentration of between 10 µM to 20 mM.

1 33. The method of claim 32, wherein said concentration is 10 mM.

1 34. The method of claim 7, wherein said target nucleic acid is single-
2 stranded DNA.

1 35. The method of claim 7, wherein the target nucleic acid is
2 comprised of RNA.

1 36. The method of claim 7, further detecting said sense transcription
2 product, said antisense transcription product, said first amplification duplex, or said
3 second amplification duplex,

4 wherein said detecting comprises hybridizing a detection oligonucleotide
5 comprising a detectable moiety, wherein said detection oligonucleotide is complementary

6 to a subsequence of said sense transcription product, said antisense transcription product,
7 said first amplification duplex, or said second amplification duplex.

1 37. A kit for copying a target nucleic acid comprising:
2 a container containing:
3 a first nucleotide primer comprising in the following order from 5' to 3':
4 a phage-encoded RNA polymerase recognition sequence,
5 a first spacer sequence comprising a sequence of from 12 to 20
6 nucleotides that consists of one nucleotide type or two
7 different nucleotide types,
8 a first target complementary sequence which can bind to a
9 segment of said target nucleic acid; and
10 a second primer comprising in the following order from 5' to 3':
11 a phage-encoded RNA polymerase recognition sequence,
12 a second spacer sequence comprising a sequence of from 12
13 to 20 nucleotides that consists of one nucleotide
14 type or two different nucleotide types, and
15 a second target complementary sequence which can bind to
16 a segment of said target nucleic acid.

1 38. The kit of claim 37, wherein said phage-encoded RNA polymerase
2 is polymerase selected from the group consisting of : a T7 RNA polymerase, a T4 RNA
3 polymerase, a T3 RNA polymerase, a SP6 RNA polymerase and a K11 RNA polymerase.

1 39. The kit of claim 38, wherein said phage-encoded RNA polymerase
2 is a mutant phage-encoded RNA polymerase that is competent to incorporate dNTPs into
3 a template nucleic acid.

1 40. The kit of claim 38, wherein said phage-encoded RNA polymerase
2 is a T7 RNA polymerase.

1 41. The kit of claim 40, wherein said T7 RNA polymerase contains a
2 Y639F mutation.

1 42. The kit of claim 40, wherein said T7 RNA polymerase contains a
2 S641A mutation.

1 43. The kit of claim 40, wherein said T7 RNA polymerase contains at least
2 two mutations.

1 44. The kit of claim 37, further comprising a member selected from the
2 group consisting of:
3 a DNA polymerase;
4 a Reverse Transcriptase that lacks RNaseH activity;
5 a phage-encoded RNA polymerase;
6 all four dNTPs;
7 those rNTPs represented in said first and second spacer sequences;
8 reaction buffer containing manganese in a concentration from 10 µM to 20
9 mM;
10 a positive control target nucleic acid; and
11 instructions for carrying out a method of copying a nucleic acid using said first
12 primer and said second primer.